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ESHRE Preimplantation Genetic Diagnosis (PGD) Consortium: data collection II (May 2000)

ESHRE PGD Consortium Steering Committee*

In 1997, the ESHRE PGD Consortium was formed as part of the ESHRE Special Interest Group on Reproductive Genetics, in order to undertake a long-term study of the efficacy and clinical outcome of preimplantation genetic diagnosis (PGD). In December 1999, the first PGD Consortium report was published discussing referrals of 323 couples, 392 PGD cycles and 82 pregnancies and 79 children born. In the second round of data collection, contributing centres were asked to send in data from their PGD activities before January 1997, as well as from 1st October 1998 until 1st May 2000, in order to have as complete as possible an overview of PGD practices in these centres. A further 563 referrals were sent in as well as 926 PGD cycles, and data on 89 pregnancies (including seven pregnancies ongoing from the previous group) and 83 children were collected. This has led to a considerable amount of cumulative data being acquired: over a period of 7 years (the oldest PGD cycle reported dates from 1994), referral data on 886 couples, cycle data on 1318 PGD cycles and data on 163 pregnancies and 162 babies were collected. In all, these data are encouraging: they show first, that the practice of PGD is becoming more and more established, and an increasing number of different applications is emerging; and second, that collecting these data is worthwhile, as they will be a valuable source of information for all those involved, e.g. in counselling patients and interacting with governmental bodies.

Key words: PGD cycle data/pregnancy and baby follow-up/ preimplantation genetic diagnosis

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Introduction

Since the first report on clinically applied preimplantation genetic diagnosis (PGD) (Handyside *et al*., 1990), the number of centres involved in PGD—as well as the number of PGD treatments—have increased year by year (Harper and Handyside, 1994; Harper, 1996). This was made possible by ever-evolving technology which made accurate chromosomal and DNA investigations possible, such as fluorescence in-situ hybridization (FISH) for sexing, aneuploidy screening and structural chromosome abnormalities (Scriven *et al*., 1998; Munné *et al*., 1999; Staessen *et al*., 1999; Van Assche *et al.*, 1999), and polymerase chain reaction (PCR), with fluorescent and multiplex PCR as the most recent developments (Findlay *et al*., 1998; Sermon *et al*., 1998; Wells and Sherlock, 1998; Dreesen *et al*., 2000) for monogenic diseases. In the first ESHRE PGD Consortium report, data from 16 different centres were collected, covering a total of referral data on 323 couples, a total of 392 PGD cycles, 82 pregnancies and 79 children born (ESHRE PGD Consortium Steering Committee, 1999). Because it is the first publication reporting on all aspects of PGD in depth and detail, this report can be regarded as a welcome source of information not only in daily contacts with patients, but also in contacts with health authorities, governments, etc. For their own purposes, the contributing centres—which are listed alphabetically in the Appendix—have access to the raw data. From the first positive reactions, it is clear that there is a real need for a yearly report of the data collected by the PGD Consortium.

Besides the prospective and retrospective collection of data on accuracy, reliability and effectiveness of PGD, the aims of the ESHRE PGD Consortium are to (ESHRE PGD Consortium Steering Committee, 1999): (i) survey availability of PGD for different conditions; (ii) initiate follow-up studies of pregnancies and children born; (iii) produce guidelines and recommended PGD protocols to promote best practice; and (iv) formulate a consensus on the use of PGD. To date, the PGD Consortium has focused on data collection on PGD cycles and pregnancies and babies, but further development of the other aims is under way.

Table I. Reproductive histories of the patients requesting preimplantation genetic diagnosis (PGD)

	0	1	2	3	4	5	⩾6	Unknown
Pregnancies	279	180	143	88	75	45	36	40
Pregnancies >28 weeks	514	216	89	18	5	1	1	42
Healthy children	676	136	20	5	0	1	0	48
Affected children	601	202	37	3	0	1	0	42
Stillborn	817	16	1	0	0	1	0	51
Spontaneous abortions	610	78	50	36	26	18	22	46
Termination of pregnancies	633	107	52	21	9	3	1	60

This table shows the couples that have had 0, 1, 2 or more previous pregnancies, etc. For example, 180 couples had had one pregnancy, 143 couples had had two pregnancies, 514 couples had had no pregnancy beyond 28 weeks, etc.

Materials and methods

Membership

Before January 2000, centres interested in becoming a member of the ESHRE PGD Consortium were asked to fill in a centre registration form containing information on current PGD practices, IVF results, cost of treatment, etc. Upon receipt, a centre code was assigned, and a centre pack containing eight forms was sent. From January 2000, registration was possible through the ESHRE web site via the Special Interest Group for Reproductive Genetics page. Once registered, the new centre again received a centre code and the contact person mentioned on the registration form was given access to the other forms of the PGD Consortium.

Data collection

For information on the content of the different forms (referral, cycle, pregnancy, baby, biopsy protocol, FISH protocol, PCR protocol), we refer to the first PGD Consortium report (ESHRE PGD Consortium Steering Committee, 1999). Before March 2000, data were collected on hard copy forms that were then sent to ESHRE Central Office. Since then, it has been possible to send in data through the ESHRE web site. Here, referrals for each couple, each cycle, pregnancy and baby have to be filled in one by one and are then sent automatically to the Steering Committee. This method allows on-line and prospective data collection. Because this method was seen as impractical especially by larger centres with a large number of cycles, the possibility was offered for the centres to fill in the blank Excel spreadsheets used by the Steering Committee for data processing directly.

Table II. Reasons for PGD

Reason	No. of patients
Genetic risk and previous TOP	247/886 (27.9)
Genetic risk and objection to TOP	390/886 (44.0)
Genetic risk and sub- or infertility	259/886 (29.2)
Genetic risk and sterilization	9/886 (1.0)
Age-related aneuploidy	48/886 (5.4)
Other	69/886 (7.8)
Unknown	19/886 (2.1)

Values in parentheses are percentages.
TOP = termination of pregnancy.

Table III. Referrals according to indication

Referral	No. of patients
Chromosomal (numerical/structural)	295
X-linked	215
Autosomal recessive	206
Autosomal dominant	151
Mitochondrial	4
Two indications	7
Unknown	8

Table IV. Referrals for chromosomal disorders

Referral	No. of patients
Structural chromosomal aberrations	
Reciprocal translocation	139
Robertsonian translocation	25
Inversion	6
Deletion	5
Aneuploidy risk	
Aneuploidy risk	75
Klinefelter syndrome	16
Sex chromosomal mosaicism	14
Male meiotic abnormalities	9
Other	2
Unknown	4

Results

Referrals

The second data collection consisted of 563 referrals, bringing the total number of referrals present in the database to 886. This report deals with the latter figure, which implies that the referrals published last year are included in this article.

From Table I it is clear that the vast majority of couples have had one or more pregnancies, though healthy children have been born in <25% of them. More than one-quarter of all couples have one or more affected children. Almost the same proportion of couples suffered from spontaneous abortion or termination of pregnancy after prenatal diagnosis.

This was reflected in the reasons for PGD, which are given in Table II. The most important reason was genetic risk and objection to termination of pregnancy (44%). The group having experienced termination after prenatal diagnosis was smaller (28%). In almost one-third of the cases (29%) the genetic indication was combined with sub- or infertility, which made IVF or intracytoplasmic sperm injection (ICSI) necessary.

As far as the indications are concerned (Tables III and IV), the same overall pattern as last year (ESHRE PGD Consortium

Table V. Referrals for monogenic diseases

Autosomal recessive	No. of referrals	Autosomal dominant	No. of referrals	X-linked	No. of referrals
Cystic fibrosis	77	Myotonic dystrophy	57	Duchenne/Becker's muscular dystrophy	52
Thalassaemia	36	Huntington's disease	44	Fragile-X syndrome	52
Spinal muscular atrophy (type 1)	31	Charcot–Marie–Tooth disease 1A	17	Haemophilia	19
Tay–Sachs disease	8	Other	33	Wiskott–Aldrich syndrome	9
Other	54			Other	83

Table VI. Centre decision[a]

Decision	Yes	No	Undecided/unknown
Suitable for IVF	639	60	187
Technically possible	637	157	92
Ethically acceptable	643	48	195
PGD accepted	565	199	122

[a]Decisions taken by PGD centres based on technical matters, etc.

Table VII. Reasons for declining PGD[a]

Reason	No. of patients
Inconvenience/burden of IVF or ICSI	41
Low success rate	24
Spontaneous pregnancy	21
Lost to follow-up	12
Undecided	11
Cost	11
Postponed	5
Other centre	5
PND instead of PGD	4
Donor oocytes needed	4
Age-related risks	1
Inaccuracy	1
Donor spermatozoa needed	1
Unknown	9

[a]Decision taken by couples.
PND = prenatal diagnosis.

Steering Committee, 1999) emerged, although some differences could be identified. The referrals for reciprocal translocations still outnumbered those for Robertsonian translocations. The only reciprocal translocation referred more than once was the frequently occurring translocation (11;22). Referrals for other structural abnormalities did not reach high numbers. Y-chromosome deletions and the 13q deletion resulting in retinoblastoma were referred more than once.

Referrals for aneuploidy screening comprised a variety of indications, among which maternal age predominates. Other reasons included in this group were failed IVF, implantation failure, and recurrent spontaneous abortion.

The referrals for monogenic disorders (Table V) showed identical patterns as in the previous report. Cystic fibrosis was the most frequent reason for referral, followed by thalassaemia and spinal muscular atrophy (type I) as far as the autosomal recessive disorders were concerned. The group of autosomal dominant diseases was dominated by the trinucleotide repeat disorders myotonic dystrophy (57 couples) and Huntington's disease (44 couples). For the Fragile-X syndrome, as well as Duchenne/Becker's muscular dystrophy, 52 couples were referred for each condition. Referrals for several other X-linked diseases were noted, though in most cases the numbers were small (with the exception of haemophilia and Wiskott–Aldrich syndrome) (Table V).

In the majority of cases the patients were suitable for IVF or ICSI, and PGD was technically possible and/or ethically acceptable (Table VI). For different reasons a total of 199 couples could not be accepted for PGD. Technical obstacles were the main reason for not being able to offer diagnosis. In a few cases this was related to the fact that diagnosis on frozen embryos was requested. Some patients were referred to a centre that had the test already available. About 9% of the patients did not fulfil the criteria for IVF or ICSI. Some patients were simply too old, while others showed a high FSH concentration. IVF was also considered risky to the mother in some cases of myotonic dystrophy and spinal muscular atrophy. One of the ethical objections was in the case of non-disclosure testing for Huntington's disease.

The reasons for declining PGD show that the largest group quit because of the burden of the procedure, followed by the low success rate. Financial aspects appeared to play a minor role. It was also interesting to note that in 21 cases, a spontaneous pregnancy was the reason for declining (Table VII).

Cycles

This year we were able to obtain almost complete data for 1318 PGD cycles. At the ESHRE PGD Consortium meeting it was decided that the aneuploidy screening data should be presented separately, as almost all of these patients were infertile, and aneuploidy screening was performed rather than a specific diagnosis. This year, no cycles involving the freezing of embryos, either before or after the biopsy, were included. From next year, these will be dealt with separately.

The data for PGD for aneuploidy screening are summarized in Table VIII. A total of 465 cycles reached the oocyte retrieval stage. All patients were infertile, and the indications included repeated IVF failure, maternal age and recurrent abortion. The majority of biopsies were performed on cleavage-stage embryos using acidic Tyrode's drilling, although 25 cycles used polar body biopsy only and one used polar body and cleavage-stage biopsy. All biopsies were performed using aspiration. All diagnoses were performed using FISH. From a total of 6025 oocytes retrieved, a fertilization rate of 62% was achieved.

Table VIII. Cycle data (*n*) for aneuploidy screening

Cycles to OR	465
IVF	123
ICSI	342
AT drilling	432
Laser drilling	11
Mechanical	22
Polar body biopsy	26[a]
Cleavage aspiration	440
COC	6025
Inseminated[b]	5432
Fertilized	3755
Biopsied	2994
Successfully biopsied	2950[c]
Diagnosed	1859
Transferable	676[d]
Transferred	908
Cycles to ET	368
Frozen	7
HCG-positive	23[e]
Positive heart beat (% per cycle)	133 (28)
Lost to follow-up	0

[a]One cycle had polar body and cleavage-stage biopsy.
[b]Number of oocytes inseminated is not accurate, as some centres did not record this information. In cycles where the data are not recorded, the figure entered was the same as the number of oocytes collected.
[c]One centre (116 cycles) did not record the number of successful biopsies, which explains that the number recorded was the same as the number of embryos biopsied.
[d]One centre (116 cycles) did not record the number of embryos diagnosed as transferable.
[e]One centre (116 cycles) did not perform an HCG test and so this value was not available.
AT = acidic Tyrode's; COC = cumulus–oocyte complex; ET = embryo transfer; HCG = human chorionic gonadotrophin; ICSI = intracytoplasmic sperm injection; OR = oocyte retrieval.

The biopsy was successful in 99% of cases, but this figure was not accurate as one centre did not record the number of successful biopsies (this centre reported a 99.2% successful biopsy rate). A diagnosis was obtained in 63% of embryos undergoing FISH. Only 36% of embryos were diagnosed as suitable for transfer, but this may be an underestimate as one centre did not record this information. This centre recorded the number of cells, fragmentation and multinucleation at days 2 and 3, as well as several other parameters. Depending on the age of the patient and previous IVF cycles, it was determined which embryos to transfer. It was interesting to note that this centre did not classify the embryos as normal or abnormal on the FISH result, but considered other factors. In all, 79% of cycles resulted in an embryo transfer, although for a number of cycles no embryos were diagnosed as transferable, and embryos were still transferred. This was reflected in the data by the fact that the number of embryos transferred was greater than the number transferable. Therefore the value entered here was an underestimate as it was recorded as the same as the number of embryos transferred. Again, this was caused by one centre using a special transfer policy, which can be explained by the fact that the main aim of aneuploidy screening is to increase the IVF pregnancy rate.

Only seven embryos were frozen. The human chorionic gonadotrophin (HCG) concentrations were not recorded by one centre, but the clinical pregnancy rate was 29% per oocyte retrieval and 36% per embryo transfer procedure.

Table IX. Summary data (*n*) of PGD cycles (not including PGD for aneuploidy)

	PCR	FISH	Total
Total cycles			853
Cancelled			82
Cycles to OR	385	386	771
IVF	35	119[a]	154[a]
ICSI	350	269	619
Cancelled post OR	8	5	13
FISH	9[b]	381	390[b]
PCR	377	0	377
AT drilling	300	302	602
Laser drilling	72	74	146
Mechanical	5	5	10
Polar body biopsy	1[c]	3	4[c]
Cleavage aspiration	377	378	755
COC	5123	5144	10 267
Inseminated[d]	4482	4608	9090
Fertilized	3140	3325	6465
Biopsied	2389	2835	5224
Successfully biopsied	2331	2710	5041
Diagnosed	1889	2434	4323
Transferable	1048	790	1838
Transferred	705	635	1340
Cycles to ET	318	321	639
Frozen	251	109	360
HCG-positive	96	78	174
Positive heart beat (% per cycle)	83	58	141 (16.5)
Lost to follow-up	0	4	4

[a]Two FISH cycles had IVF and ICSI.
[b]Nine cycles involved PCR and FISH diagnosis.
[c]One cycle PCR diagnosis had polar body biopsy and cleavage-stage aspiration, and the diagnosis involved PCR and FISH.
[d]Number of oocytes inseminated is not accurate, as some centres did not record this information. In cycles where the data are not recorded, the value entered was the same as the number of oocytes collected.
FISH = fluorescence in-situ hybridization; PCR = polymerase chain reaction. Other abbreviations as Table VIII.

The FISH and PCR diagnoses for PGD of inherited disorders are summarized in Table IX. A total of 853 cycles was started, of which 82 were cancelled before the oocyte retrieval due to a poor response, cysts or other reasons (9.6% cancellation rate). In all, 771 cycles reached the stage of oocyte retrieval. The majority of cycles had ICSI ($n = 619$), while two cycles had IVF and ICSI combined. A PCR diagnosis was performed in 377 cycles, FISH in 381 cycles, and nine cycles had FISH and PCR diagnosis combined. This included one cycle combining aneuploidy screening and a single gene defect (the cycle where polar body and cleavage-stage biopsy were combined). In five cycles sexing only was carried out using both PCR and FISH, and in three cycles a specific diagnosis of an X-linked disease was combined with sexing by FISH. All of these cycles were included under the PCR diagnosis. Thirteen cycles were cancelled after the oocyte retrieval, mainly due to insufficient quality of the embryos for biopsy. From 10 267 oocytes collected, a fertilization rate of 63% was obtained. The number of oocytes inseminated was not an accurate figure, as some centres did not record this information. From the 6465 fertilized oocytes, 81% were suitable for biopsy, of which 96% were successfully biopsied, this being consistent with the data for last year. The majority of cycles used acidic Tyrode's to drill the zona ($n = 602$), and 146 cycles used the

laser—which was an increase from last year's data. The majority of cases had cleavage-stage biopsy (755 cycles), all of which used blastomere aspiration to remove the cell. Three cycles had polar body biopsy only (all FISH diagnoses), and one cycle had polar body and cleavage-stage biopsy (PCR diagnosis).

The diagnosis was obtained in 86% of embryos successfully biopsied, and of these, 43% were diagnosed as suitable for transfer. From the number of oocytes collected, only 18% were finally diagnosed as suitable for transfer, which confirms the need for the retrieval of a high number of oocytes for a successful PGD cycle (Vandervorst *et al.*, 1998). A total of 639 cycles reached the embryo transfer stage, and 1340 embryos were transferred. In this series, 360 embryos were cryopreserved, and some have been thawed and transferred, though there has as yet been no publication reporting a pregnancy from frozen–thawed embryos.

A positive HCG was detected in 174 cycles (23% per oocyte retrieval), and 141 were confirmed as clinical pregnancies following an ultrasound scan (16.5% per started cycle, 18% per oocyte retrieval, 22% per embryo transfer procedure).

When the cycles were separated according to the diagnostic method used, the following results were obtained: PCR diagnoses were performed for a variety of autosomal recessive and dominant disorders and for sexing or specific diagnosis for X-linked diseases. For the PCR diagnosis, 385 cycles reached oocyte retrieval. It is well documented that for PCR diagnosis, fertilization should be achieved by ICSI to reduce the risk of contamination from sperm embedded in the zona pellucida, yet IVF was still used in 35 cycles. A successful PCR diagnosis was obtained in 81% of embryos successfully biopsied, and 55% were diagnosed as transferable. A pregnancy rate of 22% per oocyte retrieval and 26% per embryo transfer procedure was obtained.

FISH was used for the diagnosis of sex for X-linked disease and patients carrying Robertsonian and reciprocal translocations (see Table X). For the FISH diagnosis, 386 cycles reached the stage of oocyte retrieval. A successful diagnosis was obtained in 90% of embryos successfully biopsied, and of these only 32% were diagnosed as suitable for transfer. This was mainly due to the high numbers of abnormal embryos detected for patients carrying translocations. Table X shows the breakdown of the PGD cycles for chromosome analysis. This mainly involved patients carrying Robertsonian or reciprocal translocations. From a total of 196 cycles that reached the oocyte retrieval stage, ICSI was performed in most cases, some of which were probably because of poor sperm quality due to the man carrying the translocation. One cycle had IVF and ICSI. Three cycles were cancelled after the oocyte retrieval, probably due to insufficient embryo development. Acidic Tyrode's was used for drilling in 157 cycles. Polar body biopsy was used for three cycles, and cleavage-stage aspiration for 190 cycles. From the 2732 oocytes collected, 85% were fertilized, which was higher than for other types of PGD cycles. Of these, 85% of the embryos were considered suitable for biopsy. The embryo biopsy procedure was successful in 95% of cases, and a FISH result was obtained in 90% of embryos. Only 27% of the embryos diagnosed were considered suitable for transfer, which was just 13% of the oocytes collected. This reflects the high level of abnormal embryos detected in this group of patients (Conn *et al.*, 1998, 1999). In 19% of cycles there were no embryos suitable for transfer. A clinical pregnancy rate of 19% per embryo transfer procedure and 15% per oocyte retrieval was obtained. Due to the low numbers of embryos diagnosed as transferable, only 13 embryos were frozen from this series.

The number of embryos transferred related to the pregnancy rate is shown in Table XI. As shown last year, there is an increase in the pregnancy rate if a higher number of embryos is transferred. Last year, there were only six cycles where five or more embryos had been transferred, while this year this value increased to 34 cycles, of which 23 were for aneuploidy screening. The maximum number of embryos transferred was eight, the same maximum number as last year.

Table X. PGD for chromosomal abnormalities (*n*) such as translocations

Cycles to OR	196
IVF	45[a]
ICSI	152
Cancelled after OR	3
AT drilling	157
Laser drilling	36
Mechanical	0
Polar body biopsy	3
Cleavage aspiration	190
COC	2732
Inseminated[b]	2327
Fertilized	1722
Biopsied	1471[c]
Successfully biopsied	1393
Diagnosed	1254
Transferable	349
Transferred	308
Cycles to ET	159
Frozen	13
HCG-positive	40
Positive heart beat	30
Lost to follow-up	3

[a]One cycle had IVF and ICSI.
[b]Number of oocytes inseminated is not accurate, as some centres did not record this information. In cycles where the data are not recorded, the figure entered was the same as the number of oocytes collected.
[c]One centre recorded more embryos biopsied than number of fertilized embryos.
For abbreviations, see Table VIII.

Table XI. Summary of pregnancy rate related to the number of embryos transferred

No. of embryos transferred per procedure	No. of embryo transfer procedures	Positive fetal heart (% per embryo transfer)
1	259	39 (15)
2	402	98 (24)
3	249	99 (40)
4	64	25 (39)
⩾5[a]	34	15 (44)

[a]One pregnancy obtained after the transfer of eight embryos.

Table XII. Evolution of pregnancy

Condition	*n*		*n*
Pregnancies	163	Fetal sacs	224
Singletons	110/163 (67)		
Twins:	46/163 (28)		
Triplets	6/163 (4)		
Quadruplet	1/163 (<1)		
First-trimester pregnancy loss			
No heartbeat			15/224
Blighted ovum			1/224
Extrauterine pregnancy (one no heartbeat)			3/224
Miscarriage			7/224
Vanishing twins (three no heartbeat)			6/224
Ongoing pregnancies (⩾12 weeks)	138	Fetuses	192
Second-trimester pregnancy loss			
TOP for misdiagnosis	2/138		2/224
Second-trimester miscarriage	2/138		3/224
Stillbirth (23, 24 weeks)	2/138		2/224
Premature rupture of membranes (6 months; twin)	1/138		2/224
Reductions of multiple pregnancies (no pregnancy loss)			
Triplet → twin	(3/138)		3/224
Triplet → singleton	(2/138)		4/224
Quadruplet → twin	(1/138)		2/224
Normal evolution	131	Fetuses	174
Singletons	89/131		
Twins	41/131		
Triplet	1/131		
No follow-up	3	Fetuses	5
1 singleton, 2 twins			
Still ongoing	5	Fetuses	7
Singletons	3/5		
Twins	2/5		
Deliveries	123	Babies	162
Singletons	85/123 (69)		
Twins	37/123 (30)		
Triplet	1/123 (<1)		

Values in parentheses are percentages.
TOP = termination of pregnancy.

Table XIII. Complications of pregnancy in 95 clinical pregnancies[a]

Complication	No. of patients	Outcome
No complications	64/95	
Total complications	31/95	
AHT	4/95	2 singleton, 2 twins
AHT + bleeding	1/95	1 twin
Bleeding	4/95	3 singletons, 1 twin
Cerclage	1/95	1 singleton
Chorioamnionitis, stillborn at 23 weeks	1/95	1 singleton
Diabetes	2/95	2 singleton
HELLP	2/95	2 singletons
Idiopathic thrombocytopenia	1/95	1 singleton
IUGR	1/95	1 singleton
Polyhydramnios, miscarriage at 20 weeks	1/95	1 twin
Preterm contractions	2/95	1 singleton, 1 twin
Preterm labour	7/95	3 singletons, 4 twins
Pre-eclampsia	3/95	2 singletons, 1 twin
Eclampsia	1/95	1 twin
Premature rupture of membranes	3/95	3 twins
Toxoplasmosis	1/95	1 singleton
Complications in singletons	19/85 (22)	
Complications in twins	12/37 (32)	

Values in parentheses are percentages.
[a]No information is available on possible complications in the triplet pregnancy.
HELLP = haemolysis elevated liver enzymes low platelets; IUGR = intrauterine growth retardation.

Table XIV. Method of delivery, and gestational age

	Total	Singleton	Twin	Triplet[a]
Method of delivery				
Vaginal	55/123 (45)	45/85 (53)	10/37 (27)	0/1
Caesarean section	51/123 (42)	30/85 (35)	20/37 (54)	1/1
Unknown	17/123 (13)	10/85 (12)	7/37 (19)	0/1
Gestational age at delivery				
Preterm	28/123 (23)	8/85 (9)	19/37 (51)	1/1
At term	88/123 (70)	74/85 (87)	14/37 (38)	0/1
Unknown	7/123 (7)	3/85 (4)	4/37 (11)	0/1

Values in parentheses are percentages.
[a]No information is available on possible complications in the triplet pregnancy.

Pregnancies

Up to May 2000, data on 163 pregnancies and 224 fetal sacs were collected, the oldest of which dated from 1993. Thirty-two of the 224 fetal sacs were lost during the first trimester, leading to 138 pregnancies which went on to the second trimester. The details of the evolution of the pregnancies is given in Table XII. During the second trimester, seven pregnancies were lost, two most notably by termination after misdiagnosis discovered at prenatal diagnosis. Five triplet and one quadruplet pregnancies were reduced to one singleton and five twin pregnancies. A total of 131 pregnancies progressed, five of which were ongoing by May 2000, three for which no further information was available, and 123 which had delivered (85 singletons, 37 twins and one triplet). The reason why so few ongoing pregnancies were recorded is that centres were asked to send in pregnancy and baby data when the pregnancy was completed, either through birth, miscarriage, or other reasons.

Complications of pregnancy are shown in Table XIII for 95 pregnancies for which complete information was available. Although, for reasons of scientific correctness, the pregnancies where the 'complications' field was left blank ($n = 18$) were not included in the calculations, it can be assumed that most of these pregnancies were without problems. No complications were reported in 64 pregnancies, but complications did occur in 31 pregnancies, with more than one complication reported for some cases. An important proportion of these complications (preterm labour, premature rupture of membranes) originated from multiple pregnancies, as is also illustrated by the rate of complications (calculated on the totality of deliveries) in singletons (22%) as compared with twins (32%).

Not surprisingly, Table XIV shows a higher level of prematurity (51% for twins compared with 9% for singletons) and delivery by Caesarean section (54% for twins compared with 35% for singletons) in the twins.

Babies

Data reported on 162 live-born children are shown in Table XV. The sex ratio was heavily skewed towards female children due to the high number of girls born after sexing for X-linked

Table XV. Data on live-born children

Parameter		Not available
Total children born (*n*)	162	
Sex		
Female	94/150	12/162
Male	56/150	17/162
Birth weight (g)	Mean 2824 ($n = 145$)	
Singletons	Mean 3167 ($n = 81$)	
Twins	Mean 2344 ($n = 64$)	
Birth length (cm)	Mean 47.9 ($n = 93$)	69/162
Head circumference (cm)	Mean 33.2 ($n = 60$)	102/162
Apgar scores	Good in 78/82	80/162
	Bad in 4/82	
	1 premature child (24 weeks); neonatal death	
	1 term child; 24 h neonatal observation	
	1 ASD, dysmature twin, neonatal ward < 24 h, good evolution	
	1 slightly premature twin, good evolution	
Malformations at birth	None in 121/130	32/162
	Malformations in 9/130	
With good evolution (one each of): ASD, bilateral clubfoot, congenital hip luxation, pes equinovarus, cystic mass in abdomen, mongolian spot, sacral dimple		
With neonatal death (one each of): exencephaly, chylothorax		
Neonatal complications	None in 74/130	32/162
	Present in 56/130	
	49/130 premature	
	9/49 prematurity + complication	
	3 neonatal observations	
	2 artificial respiration	
	1 PDA; normal evolution	
	3 neonatal deaths:	
	1 intracranial bleeding, 1 exencephaly, 1 chylothorax	
	4/130 dysmature	
	1/130 3 days neonatal feeding in infant with low birth weight	
	1/130 respiratory problem	
	1/130 neonatal observation in term baby because of bad Apgar	

ASD = atrial septal defect; PDA = patent ductus arteriosus.

diseases. The average birth weight for 145 children was 2824 g, with an average of 3167 g for 81 singletons and 2344 g for 64 twins. The average birth length was 47.9 cm ($n = 93$), and the average head circumference was 33.2 cm ($n = 60$). Apgar scores were good ($\geqslant$8 at birth) in 78 out of 82 children and bad ($<$8 at birth) in four children, three of whom showed a good further evolution and one of whom (a severely premature child) died.

Information about the presence or absence of malformations was available for 130 children; 121 of these showed no malformation. (Again, it can be assumed that the 32 for which no information was given did not show malformations.) Seven children showed non-life-threatening malformations ranging from mongolian spot to bilateral clubfoot, and two children died due to severe malformations (exencephaly and chylothorax). No information on possible surgical correction was available as this type of information was not requested.

Confirmation of diagnosis

In total, 116 of the 236 fetal sacs (49%) were examined through prenatal diagnosis (Table XVI). Unfortunately, four cases were shown to be misdiagnosed at PGD. Two of these pregnancies (one affected with myotonic dystrophy and one with β-thalassaemia) were terminated, while the two other [one with cystic fibrosis, and one male fetus after sexing for X-linked retinitis pigmentosa (RP)] went on to term. Whether this boy was affected with RP is not known. In Table XVI, the obvious confirmation of PGD for sexing by the baby's sex at birth is not taken into account. After PGD for sexing, only one misdiagnosis (see above), which was discovered at prenatal examination occurred after preimplantation sex determination using PCR. In four early miscarriages a karyotype was obtained: two miscarriages showed an abnormal karyotype (one trisomy 16 and one mosaic trisomy 22). Although both abnormal karyotypes occurred in the FISH group, these cannot be classified as misdiagnoses as the chromosomes involved were not examined at PGD.

Protocols

In this round of data collection, very few centres ($n = 4$) contributed data. This may have been for a number of reasons: (i) difficulties in accessing the web-based submission site and accessing the posted entries; (ii) voluntary nature of protocol submission; and (iii) many centres had previously sent data

Table XVI. Confirmation of diagnosis

Method used	Prenatal method	*n*	Result	*n*	Postnatal method	*n*	Result	*n*	Total
FISH	CVS	18			Karyo miscarriage	3	Normal	1	72/129 fetal sacs tested
	Amnios	44					Abnormal	2[a]	
	Unknown	4			Karyo postnatal[b]	4	Normal	4	
	Total	66	Normal	63/66	Total	7	Normal	5	
			Unknown	3/66			Abnormal	2	
PCR	CVS	27			Genetic testing[c]	10	Normal	10	60/107 fetal sacs tested
	Amnios	21			Sweat test[d]	5	Normal	5	
	Unknown	2			Miscarriage	2	Normal	2	
	Total	50	Normal	46	Total	17	Normal	17	
			Abnormal	4[e]			Abnormal	0	
	Total prenatal testing		Total result		Total postnatal testing		Total postnatal result		
		116/236	Normal	109/116		24/236	Normal	22/24	132/236 fetal sacs tested
			Abnormal	4/116			Abnormal	2/24	
			Unknown	3/116					

[a]Trisomy 16, mosaic trisomy 22.
[b]3×first testing, 1 confirmation.
[c]4×first testing, 6×confirmation.
[d]4×first testing, 1 confirmation.
[e]One affected myotonic dystrophy, one affected β-thalassaemia, one affected cystic fibrosis, one 46,XY after PCR sexing for X-linked retinitis pigmentosa.
CVS = chorionic villus sampling.

and might be unwilling to repeat the process if it was only slightly changed from the previous submission.

Discussion

Referrals

The first data set contained almost as many referrals as treatment cycles. It is clear that the first data collection was biased towards the couples who underwent treatment. In the second set, some centres have provided all the referrals since the start of their PGD activities, irrespective of treatment and outcome. This is most likely the reason for some of the differences observed.

With respect to the reproductive histories of the patients requesting PGD, and the reasons for PGD, there are few differences between the two data sets: as shown above, most of these patients have a reproductive history burdened with pregnancy terminations and/or affected children.

If the broad indication groups are considered (see Table III), it is clear that a chromosomal indication is becoming an increasingly important reason for referral. This is most likely a reflection of technical improvements regarding FISH. In principle, there are now probes available for almost all structural abnormalities, and the simultaneous applications of several probes is becoming more or less routine. The number of referrals must have been biased negatively with respect to aneuploidy screening in couples having an indication for IVF or ICSI. From the literature it is known that some centres have reported a large number of treatment cycles just for this indication, and therefore it is surprising that this category of referrals amounted to $<10\%$ of the total. The majority of these screenings are related to maternal age.

With respect to the monogenic diseases, no dramatic differences compared with last year's data collection were noted. Cystic fibrosis was the first Mendelian disorder to be diagnosed (Handyside *et al*., 1992), and after 10 years still shows the highest number of referrals. The group of trinucleotide repeat disorders (myotonic dystrophy, Huntington's disease and Fragile-X syndrome) follows, with an average of 50 couples per disease.

In general, it might be concluded that the pattern of referral indications is more or less a reflection of the genetic disorders requiring prenatal diagnosis. One of the differences that is clearly present is the number of referrals with the combination of two genetic disorders segregating at the same time. A total of seven couples presented with this phenomenon. For the time being, it will be very difficult to help these couples, but as soon as multiple diagnoses become technically feasible it is to be expected that the number of embryos available for transfer might become the limiting factor. In contrast, prenatal diagnosis is less of a problem from a technical point of view, but the chance of finding an abnormality requiring termination is increased so much that this is not a workable alternative.

The decisions taken by the centres after referral are a reflection of the status of the group of couples from the second and thus later data collection, since in about 20% of the cases the decision-making process on the possibility and acceptability of PGD is not yet finished.

The PGD Consortium is aiming at the collection of complete and prospective referral data, which might assist in taking decisions with respect to the developments of new diagnostic procedures according to needs emerging from the data collected. Furthermore, in future some Consortium members might decide to concentrate only on the diagnosis of rare disorders in their centres, while other centres would then refer their patients to them.

Cycles

Comparing data from last year and this year, a number of interesting factors can be seen, such as the increase in the use of the laser for zona drilling and a number of polar body biopsy cases. From the data available, there appears to be no difference in pregnancy rates between cycles in which acidic Tyrode's solution or laser technology has been used. No blastocyst biopsy for PGD has yet been reported to the PGD Consortium. On the diagnosis side, unfortunately there are still centres using IVF for PCR diagnosis, and it is hoped that in the future all centres will use ICSI to avoid sperm contamination. Although comparing the number of cycles from the first and second report for each type of diagnosis is awkward (as the reports cover very different periods in time), an attempt can still be made. The total number of cycles reported has increased tremendously, mostly due to the larger number of contributing centres. This is also reflected in a clear increase in numbers of PCR cycles reported. For FISH analysis, the relative numbers of aneuploidy screening, chromosomal aberration cases and sexing cases can be compared: the number of aneuploidy screening cases has increased three-fold, the number of chromosomal aberration cases has increased by a factor of four, while the number of sexing cases has decreased slightly. This reflects the evolution in current PGD practice: while the number of centres using aneuploidy screening to improve their IVF results is steadily increasing, and while the number of patients with translocations and other chromosomal aberrations which can be helped by PGD is also increasing due to technical improvements, an increasing number of X-linked diseases can be diagnosed by a DNA-specific test which obviates the need for sexing. Another striking observation, which has been commented upon (Conn *et al*., 1998, 1999), is the low number of embryos available for transfer after PGD for chromosomal aberrations. The underlying mechanisms of this phenomenon are a worthwhile research subject.

With regard to pregnancy rates, no evolution is observed between the first and second sets of data. Pregnancy rates are quite similar, and remain at ~17% fetal heart beats per cycle started. Again, the time periods which these two data collections cover cannot be used for comparison, but finer analysis of the available data is certainly a possibility which the ESHRE PGD Consortium will pursue.

Due to the complexity and the large amount of data, the steering committee decided this year to show only summarized data in this paper. However, the steering committee of the ESHRE PGD Consortium has been expanded so that more people can be involved with the analysis of this large amount of data in the future, which will hopefully lead to the reporting of more detailed information.

Pregnancies

The first fact to come to attention when examining Table XII is the high rate of multiple pregnancies (33%), in contrast to the moderate pregnancy rate per cycle (16.5%). Although several publications have now shown that careful selection of one or two viable embryos for transfer is effective in reducing multiple pregnancies (Staessen *et al*., 1995; Gerris *et al*., 1999), this type of selection is not easily applicable in PGD. First, it is still unclear to what extent biopsy of one or two cells from an embryo impairs the implantation potential. Second, at each PGD a cohort of embryos is diagnosed as unsuitable for transfer on genetic grounds, while this cohort could well contain the embryos with the highest implantation potential. Third, PGD embryos are transferred at day 3 or 4, while most IVF centres now transfer embryos in standard ICSI patients at day 2, or sometimes at day 5 at the blastocyst stage, which makes comparing pregnancy rates after PGD and ICSI difficult. Clearly, the PGD Consortium data collection would be an ideal tool for investigating what selection criteria apply to embryos post biopsy. Tables XIII and XIV simply confirm the high rate of multiple pregnancies and the complication during pregnancy and at birth which this entails. The incidence of pregnancy loss [subclinical pregnancies (i.e. pregnancies with a positive HCG, but no fetal heart beat), clinical abortions and extrauterine pregnancies] was 32/163 (20%), which is comparable with a value of 22.4% referred to by others (Wisanto *et al*., 1995) for ICSI with ejaculated spermatozoa. However, caution is mandatory because the retrospective nature of the data collection may lead to underestimation of chemical pregnancies. Reassuringly, no specific complication emerges which could be linked to PGD.

Babies

The cohort of 162 children described here is very similar to a cohort of 1987 children born after 'regular' ICSI (Bonduelle *et al*., 1999): 52 and 54% were singletons, 46 and 41% were twins, and 2 and 5% were triplets respectively. Other parameters such as birth weight were also very similar: singletons weighed 3176 and 3220 g, and twins weighed 2344 and 2421 g respectively. Birth length and head circumference were equally similar. When we apply the definition of major malformation used in this publication (i.e. malformations that generally cause functional impairment or require surgical correction), we obtain a rate of 3/130 (bilateral clubfoot, exencephaly and chylothorax) or 2.3%. Again, this is very close to the 2.9% obtained by others (Bonduelle *et al*., 1999). Although data on only a small number of cases are available as yet, an important message to emerge here—and which has been one of the first concerns of the ESHRE PGD Consortium—is that PGD babies are not exposed to greater risks of neonatal problems or malformation than ICSI babies.

Confirmation of diagnosis

Another important aim of the ESHRE PGD Consortium is to assess the accuracy of PGD. In this respect, it is assuring to see that >50% of the concepti were checked before or after birth. Less assuring is that four misdiagnoses for monogenic diseases occurred by PCR, which underscores the greater technical difficulties encountered with PCR than with FISH. One of these misdiagnoses was probably due to contamination during PCR; for the other three, no explanation was given or available, although it would be interesting to know why these misdiagnoses occurred in order to prevent such events in the future, possibly through guidelines issued by the PGD Consortium. Besides diagnosis based on two biopsied cells (as

is already applied by a number of centres), application of recent technical developments such as multiplex PCR (Kuliev *et al*., 1999; Dreesen *et al*., 2000) may decrease this misdiagnosis rate of 4/116 (3.4%). This emphasizes the need for the ESHRE PGD Consortium to advise a control prenatal diagnosis after PGD, which must still be regarded as an experimental procedure.

Protocols

As an alternative to the current way of collecting data concerning protocols, the steering committee has decided to set up retrospective studies in different technical areas applied in PGD. The consortium could rapidly prove valuable in the recommendation of best practice guidelines to new and existing PGD centres, using information gained from each of the participating centres. In many centres, the low number of cycles performed annually prohibits the collection of data from which meaningful generalizations can be made. Data from the consortium potentially provides power to comparisons by pooling equivalent data sets. The comparisons would mostly be performed retrospectively and should as far as possible attempt to compare like with like. These studies will be loosely divided between three main areas in line with the existing questionnaires (i.e. biopsy/culture; FISH testing; PCR testing). Each of these areas could later be further subdivided, e.g. FISH-related studies could be divided into translocations, sexing and aneuploidy screening. The Consortium has appointed task force leaders for each of these areas whose responsibilities include data collection, analysis and publication. These studies should neither interfere with clinical practice, compromise innovative work nor replace publications describing novel work. At the present time, the proposals for retrospective studies include the following:

1. Biopsy/culture/IVF-related studies
   (i) Outcome of embryos biopsied at 4- to 6-cell stage on day 3.
   (ii) Outcome of cryopreservation of biopsied embryos (with respect to method of biopsy and developmental stage at which embryos are cryopreserved)
   (iii) Use of Ca/Mg-free biopsy medium
   (iv) Developmental potential of embryos (following biopsy of one or two cells)
   (v) Effect of day of embryo transfer on PGD pregnancy rates
2. FISH-related studies
   (i) IVF versus ICSI as fertilization method for FISH cases
   (ii) Optimal number and choice of probes for FISH sexing/aneuploidy screening
   (iii) Accuracy of FISH diagnoses (following biopsy of one or two cells)
   (iv) Outcome of translocation cases
3. PCR-related studies
   (i) Comparison of allele dropout rates using different lysis buffers/methods
   (ii) Accuracy of PCR diagnoses (following biopsy of one or two cells)
   (iii) Recommended cell numbers and types for clinical PGD assay development
   (iii) Recommended number of contamination controls for work-up and clinical cases

Conclusions

The PGD Consortium has clearly shown that the practice of PGD is becoming increasingly established, while a still wider range of applications is emerging. This encourages us to continue data collection and to attempt completion and correction of currently collected data. These data will be a valuable source of information, for example for all those involved in counselling patients and interacting with governmental bodies.

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